

Anaerobic Biofilm Infections in Cystic Fibrosis

Pseudomonas aeruginosa is an important pathogen that infects the lungs of cystic fibrosis patients. A paper published in the October issue of *Developmental Cell* (Yoon et al., 2002) proves that the bacterial cells in the lung live in anaerobic biofilms and adopt a metabolic pattern and a phenotype that differ significantly from those of test tube-grown cells, and even from those of aerobic biofilms.

A recent paper from a team led by Dan Hassett (Yoon et al., 2002) will profoundly change the way we visualize and confront *Pseudomonas* infections in the lungs of patients with cystic fibrosis (CF). The paper epitomizes an exciting new approach in modern medical microbiology, recognizing that bacteria are highly “plastic” in their phenotypic response to changing environmental conditions, and resolves to define the phenotype actually present in the infection being studied. The first steps in this direction were the demonstration (Singh et al., 2000) that *P. aeruginosa* grow in biofilms (Stoodley et al., 2002) in the CF lung and that the microbial environment of the CF lung is essentially anaerobic (Worlitzsch et al., 2002). Yoon et al. go on to examine the ability of this species to form biofilms in an anaerobic milieu. The results show not only that the bacteria can grow in anaerobic conditions but also that this is the preferred mode of growth.

The next logical question becomes what enables these “aerobic” organisms to thrive without oxygen using nitrate and nitrite as electron acceptors. It is useful to note that the denitrification pathway yields eight electron equivalents of energy. This goes a long way toward explaining the relative metabolic success of cells deep within biofilms, especially those in matrix-enclosed micro-colonies in the CF lung. These heterotrophic cells live entirely anaerobically in a nitrate-sufficient environment. One secondary effect of this metabolic strategy is the buildup of NO, which is toxic to the cells, so it must be removed by various enzymatic mechanisms or by inhibition of its formation. This role for NO in a “suicide” phenomenon in anaerobic biofilms was discovered by the use of live/dead stains and confocal microscopy, and its mechanism was elucidated by the imaginative examination of various signaling mutants. Specifically, regulation of NO reductase by the rhl quorum sensing (QS) system is important to keep NO levels low during anaerobic respiration. The revelation of how the cells of a putative aerobe can grow without oxygen, in the depths of these complex sessile communities, constitutes a major conceptual advance in our general understanding of biofilms.

Having shown that cells of *P. aeruginosa* grow luxuriantly in anaerobic biofilms, the authors undertake a proteomic analysis of the changes in gene expression associated with growth in anaerobic biofilms. The radical changes that characterize adaptations to the biofilm mode of growth (see also Sauer et al., 2002), and to anaerobic metabolism, are as varied as they are unexpected. One gene product that is turned on exclusively in anaerobic biofilms, Opr F, is a channel-forming porin,

which is involved in cell shape regulation and in growth in low salt. This gene might not have been expected to be upregulated in anaerobic biofilms, but its expression is seen to be increased ~40-fold. The authors go on to demonstrate that it may be involved in the transport of nitrogenous electron acceptors, or perhaps in the stabilization of enzymes of the denitrification pathway, and the whole thing seems to make sense once again. This metabolic role of cell envelope components is intellectually satisfying, because it reveals the whole bacterial cell as a well-coordinated and fully integrated metabolic “machine.”

The authors point out that our therapeutic strategies in dealing with any specific disease are dictated and limited by our concept of the mode of growth of the causative organisms. This new perception of the growth of *P. aeruginosa* in anaerobic biofilms in the CF lung will open up new therapeutic strategies based on the sensitivity of rhl QS mutants to NO suicide, because blockers of the rhl signal will induce this natural killing of biofilm cells. The new recognition of the importance of the Opr F porin to the formation of these pathogenic communities may provide equally useful therapeutic strategies. The authors remark on the possible success of Opr F vaccines, and they speculate that this outer membrane protein may constitute a very sensitive biofilm-specific target for whole new classes of antibiotics. But I believe that the consequences of their pivotal work may be even more far-reaching. Consider the clinical management of CF patients in which we assess the efficacy of high doses of the antibiotic tobramycin by sputum cultures. If the bulk of *P. aeruginosa* cells in the CF lung are growing in anaerobic biofilms, and tobramycin is ineffective in an anaerobic milieu, this antibiotic may only kill planktonic cells released from the main biofilms and never affect the biofilms themselves. This therapy may look more effective than it really is if planktonic aerobic bacteria in the sputum grow on the plates (in air), and the majority of anaerobic cells growing in the biofilm phenotype simply fail to grow in this very artificial environment. PCR data keyed to the Opr F porin would tell us the bacterial “load” of anaerobic biofilm cells of *P. aeruginosa* in patients’ sputum, and some convenient variation of the live/dead stain would tell us what proportion of these cells had survived antibiotic treatment. These modern techniques may lead us to existing agents that are effective in killing bacteria in anaerobic biofilms, and we can use them while we wait for novel antibacterials based on this new perception that *P. aeruginosa* cells grow in anaerobic biofilms in the CF lung.

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Selected Reading

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Replicating Past Lesions in DNA

Replication of damaged DNA is essential in all organisms and is potentially achieved by several mechanisms. How *Escherichia coli* employs these different mechanisms to effect efficient, accurate replication of a damaged template is revealed in this issue of *Molecular Cell*.

The inherently unstable nature of DNA, coupled with the barrage of both exogenous and endogenous mutagens it is inevitably exposed to, has led to the evolution of multiple enzyme systems capable of recognizing and removing the offending adducts. However, some damage inevitably slips past the net. The dangers posed by these lesions lie in their ability to block advance of RNA polymerases, inhibiting transcription of the damaged gene and of replicative DNA polymerases, preventing chromosomal duplication. Recent work has highlighted the important role of repair enzymes that target RNA polymerases stalled at damaged bases in the template strand. However, the mechanisms by which a cell deals with replication of damaged DNA have remained obscure. This is in spite of the discovery almost forty years ago that *Escherichia coli* cells deficient in the RecA strand exchange protein, an activity central to recombination reactions, were exquisitely sensitive to radiation damage (Clark and Margulies, 1965). Subsequent work demonstrated that gaps appeared in newly synthesized DNA strands formed from damaged template DNA, presumably due to DNA polymerase blockage by the damage, and that exchanges occur between the gapped newly synthesized strands and the parental DNA to effect repair of the gaps (Ganesan, 1974; Rupp et al., 1971). Thus, postreplication repair of DNA was first postulated and offered an explanation as to how cells could replicate damaged DNA in an inherently accurate manner via recombination. Elegant studies of RecA activity in vitro lent further weight to these models (West et al., 1981).

The discovery of specialized DNA polymerases which could replicate past lesions in DNA added another layer of complexity (Friedberg et al., 2002). These appear to have evolved to insert the appropriate nucleotide opposite specific sites of DNA damage (termed translesion replication) that would otherwise block progression of the highly accurate replicative polymerases. However, each translesion polymerase appears to operate with reasonable accuracy only at specific DNA lesions such as UV light-induced pyrimidine dimers. At other lesions, or on undamaged template DNA, their fidelity is greatly reduced. The use of these polymerases to effect replica-

tion past sites of DNA damage therefore runs the risk of enhanced mutation.

These multiple mechanisms of ensuring that chromosomal duplication can proceed on a damaged template reflect not only the necessity to complete replication but also the frequency with which the replication machinery encounters damaged DNA. However, given that multiple mechanisms do exist and that some are potentially mutagenic while others are not, how does a cell regulate which system will operate at which lesion? In this issue of *Molecular Cell*, Berdichevsky et al. (2002) address this crucial question by asking whether an abasic site located in a gap within an otherwise duplex plasmid DNA is replicated via a recombinational mechanism or via a translesion DNA polymerase in *E. coli*. They provide the first direct in vivo evidence that gaps in DNA can be repaired by recombination, finally confirming models first postulated more than 30 years ago. Furthermore, they analyzed the balance between recombinational repair and translesion synthesis. Transformation of the damaged plasmid into cells resulted in its repair primarily by recombination rather than translesion replication, even when the levels of translesion polymerases were elevated by exposing the cells to UV light prior to their transformation. Only when there was no intact homologous plasmid available for recombination did translesion replication dominate. This dominance of recombination in vivo was recapitulated in vitro by reconstituting both RecA-catalyzed strand exchange and translesion replication by DNA polymerase V in the same reaction. When the level of RecA was in excess of that of DNA polymerase V, as in vivo, then recombination dominated the reaction.

Thus, at least in *E. coli*, recombinational repair of gaps appears to be preferred over less accurate translesion replication. So why might translesion replication be required at all? The answer to this is not known. However, DNA polymerase V is one of the last enzymes to be expressed as part of the SOS damage response in *E. coli* and is responsible for the elevated mutagenesis observed during this response. This observation implies that translesion replication may be used as a last resort when the recombinational system simply cannot cope with high levels of DNA damage (Rangarajan et al., 2002). In addition, there may be types of damage that cannot be repaired by recombination.

While the work by Livneh and colleagues is a welcome beginning, many questions remain unanswered. *E. coli* encodes two other damage-inducible DNA polymerases (II and IV), and just how the cell coordinates the activities of all these polymerases, together with DNA polymerases I and III required for normal replication, is a mystery. Moreover, the consensus view is that not all template damage can be skipped over by the replication machinery to leave gapped DNA behind the advancing fork. Only lesions affecting the lagging strand template are